

0230-0162P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/1856717

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

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|-------------------------------|---------------------------|-----------------------|
| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
| PCT/JP99/06617 | November 26, 1999 | November 27, 1998 |

TITLE OF INVENTION

γδT CELL IMMUNOACTIVITY ENHANCERS CONTAINING EXTRACT OF LENTINUS EDODES MYCELIUM

APPLICANT(S) FOR DO/EO/US

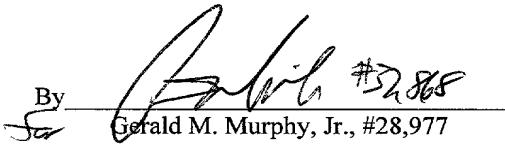
ASANO, Kenji; MATSUDA, Yukiko and TAJIMA, Yutaka

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau. WO 00/32213
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US).
6. An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith.
 - b. has been previously submitted under 35 U.S.C. 154(d)(4)
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98-1449 and International Search Report w/ cited references
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A **FIRST** preliminary amendment.
14. A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. A substitute specification.
16. A change of power of attorney and/or address letter.
17. A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. Other items or information:
PCT/ISA/210
PCT/IPEA/409
PCT Request

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| U.S. APPLICATION NO (if known, see 37 CFR 1.5) 09/856717 | INTERNATIONAL APPLICATION NO PCT/JP99/06617 | ATTORNEY'S DOCKET NUMBER 0230-0162P | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. \$1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT = Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)). <table border="1"> <tr> <td><input type="checkbox"/> CLAIMS</td> <td>NUMBER FILED</td> <td>NUMBER EXTRA</td> <td>RATE</td> </tr> <tr> <td>Total Claims</td> <td>15 - 20 =</td> <td>0</td> <td>X \$18.00</td> </tr> <tr> <td>Independent Claims</td> <td>3 - 3 =</td> <td>0</td> <td>X \$80.00</td> </tr> <tr> <td colspan="2">MULTIPLE DEPENDENT CLAIM(S) (if applicable)</td> <td>Yes</td> <td>+ \$270.00</td> </tr> <tr> <td colspan="4" style="text-align: center;">TOTAL OF ABOVE CALCULATIONS = \$ 1,260.00</td> </tr> <tr> <td colspan="4"> <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. </td> </tr> <tr> <td colspan="4" style="text-align: center;">SUBTOTAL = \$ 1,260.00</td> </tr> <tr> <td colspan="4"> Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)). </td> </tr> <tr> <td colspan="4" style="text-align: center;">TOTAL NATIONAL FEE = \$ 1,260.00</td> </tr> <tr> <td colspan="4"> Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property + </td> </tr> <tr> <td colspan="4" style="text-align: center;">TOTAL FEES ENCLOSED = \$ 1,260.00</td> </tr> <tr> <td colspan="2" rowspan="2"></td> <td>Amount to be: refunded</td> <td>\$</td> </tr> <tr> <td>charged</td> <td>\$</td> </tr> </table> | | <input type="checkbox"/> CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | Total Claims | 15 - 20 = | 0 | X \$18.00 | Independent Claims | 3 - 3 = | 0 | X \$80.00 | MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | Yes | + \$270.00 | TOTAL OF ABOVE CALCULATIONS = \$ 1,260.00 | | | | <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. | | | | SUBTOTAL = \$ 1,260.00 | | | | Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | TOTAL NATIONAL FEE = \$ 1,260.00 | | | | Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property + | | | | TOTAL FEES ENCLOSED = \$ 1,260.00 | | | | | | Amount to be: refunded | \$ | charged | \$ | CALCULATIONS | PTO USE ONLY |
| <input type="checkbox"/> CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Total Claims | 15 - 20 = | 0 | X \$18.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Independent Claims | 3 - 3 = | 0 | X \$80.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | Yes | + \$270.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL OF ABOVE CALCULATIONS = \$ 1,260.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUBTOTAL = \$ 1,260.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | | Amount to be: refunded | \$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | charged | \$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| a. <input checked="" type="checkbox"/> A check in the amount of \$ 1,260.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-2448</u> . | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Send all correspondence to: Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292 P.O. Box 747 Falls Church, VA 22040-0747 (703)205-8000 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Date: <u>May 25, 2001</u> By <u>Gerald M. Murphy, Jr., #28,977</u> #32868  /rem | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

09/856717

JC18 Rec'd PCT/PTO 25 MAY 2001

PATENT
0230-0162P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: ASANO, Kenji et al. Conf.:

Int'l. Appl. No.: PCT/JP99/06617

Appl. No.: NEW Group:

Filed: May 25, 2001 Examiner:

For: $\gamma\delta$ T CELL IMMUNOACTIVITY ENHANCERS
CONTAINING EXTRACT OF LENTINUS
EDODES MYCELIUM

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION

Assistant Commissioner for Patents
Washington, DC 20231

May 25, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP99/06617 which has an International filing date of November 26, 1999, which designated the United States of America and was not published in English.

IN THE CLAIMS:

Please amend the claims as follows:

9. (Amended) The $\gamma\delta T$ cell activity enhancer of claim 1 used for treating tumor.

10. (Amended) The $\gamma\delta T$ cell activity enhancer of claim 1 used for preventing or treating a bacterial infection or a viral infection.

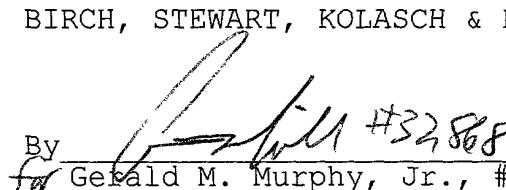
REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have been amended to delete improper multiple dependencies and place the application into better form for examination.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 
for Gerald M. Murphy, Jr., #28,977

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

(Rev. 02/12/01)

GMM/rem
0230-0162P

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

9. (Amended) The $\gamma\delta$ T cell activity enhancer of [any one of claims 1 to 8] claim 1 used for treating tumor.

10. (Amended) The $\gamma\delta$ T cell activity enhancer of [any one of claims 1 to 8] claim 1 used for preventing or treating a bacterial infection or a viral infection.

SPECIFICATION

γδT CELL IMMUNOACTIVITY ENHANCERS CONTAINING
EXTRACT OF LENTINUS EDODES MYCELIUMFIELD OF THE INVENTION

5 The present invention relates to the development and preparation of γδT cell activity enhancers and therefore immunopotentiators containing an extract of Lentinus edodes mycelium.

10 The present invention also relates to the development and preparation of foods, drinks and feeds containing an extract of Lentinus edodes mycelium and having a γδT cell activity-enhancing effect and therefore an immunopotentiating effect.

15 The present invention also relates to the development and preparation of antitumor agents, therapeutic agents against bacterial infections and therapeutic agents against viral infections containing an extract of Lentinus edodes mycelium.

20 PRIOR ART

Characteristics of γδT cells

Peripheral T cells in animal blood are mainly classified into two groups based on the type of their cell surface antigens referred to as T cell receptors (TCR).

25 One type is an αβT cell bearing TCR α and β chains on their cell surfaces, and the other is a γδT cell bearing TCR γ and δ chains. γδT cells are cytotoxic killer cells present at a level of only a few to about 10% in normal peripheral blood

and lymphoid tissue in humans, and the characteristics of which are quite distinct from those of $\alpha\beta$ T cells.

In humans, $\gamma\delta$ T cells are present in the intestinal tract, skin and peripheral blood or the like and elicit a local immunity. So far, $\gamma\delta$ T cells have been reported to have functions such as cytotoxicity against cancer cells, and protective activity against bacterial or virus infections, etc.

10 Phylaxis activity of $\gamma\delta$ T cells

Some $\gamma\delta$ T cells in the spleen and other organs produce cytokines such as IL-4 or IFN- α in response to infection. It has been shown under experimental conditions that when these cells are lacking, resistance to bacterial infections is reduced. For example, there is one report which describes that resistance to *Mycobacterium tuberculosis* infection decreased in mice treated with a $\gamma\delta$ -type TCR antibody to transiently inhibit functions of $\gamma\delta$ T cells or mice deficient in the TCR γ gene (Ladel C. et al., Eur. J. Immunol., 1995, 25:2877-2881). Another report describes that $\gamma\delta$ T cells appear during the early stage of infection with *Listeria monocytogenes* (Hiromatsu K. et al., J. Exp. Med., 1992, 175:49-56). These findings suggest that $\gamma\delta$ T cells play an important role in protecting against bacterial infections.

It has also been reported that chronic hepatitis B virus infection induces the growth of $\gamma\delta$ T cells in the liver and spleen (Ozaki S. et al., J. Med. Invest., 1998,

44:215-217); and that vaccinia virus markedly increases during the early stage of infection in $\gamma\delta$ T cell-deficient mice as compared with normal mice (Welsh RM, et al., Immunol. Rev., 1997, 159:79-93). These findings suggest 5 that $\gamma\delta$ T cells act not only on bacterial infections but also on viral infections.

Cytotoxicity of $\gamma\delta$ T cells against cancer cells

$\gamma\delta$ T cells are a class of T cells that are capable of 10 specifically targeting and killing autologous cancer cells, but show no cytotoxicity to autologous normal lymphocytes (such as $\alpha\beta$ T cells). In this respect, in cancer therapy which employs activated $\gamma\delta$ T cells there is very little danger of side effects. In contrast to $\gamma\delta$ T cells, $\alpha\beta$ T 15 cells are known to kill autologous leukocytes rather than autologous cancer cells, and thus in cancer therapy which employs activated $\alpha\beta$ T cells there is a likelihood that serious side effects will occur. In view of this, cancer therapy which employs activated $\gamma\delta$ T cells is desirable.

20 Moreover, $\gamma\delta$ T cells have similar characteristics to NK cells such as their MHC-nonrestricted cytotoxicity against cancer cells. $\gamma\delta$ T cells are present in peripheral blood of children at about 10% but decrease with age. This suggests that the increase in the occurrence of cancer with 25 age may be related to a decrease in $\gamma\delta$ T cells. In peripheral blood of chicken, sheep, cow or the like, $\gamma\delta$ T cells are found at levels as high as 15-50%. The low incidence of tumors in these animals suggests that the

presence of peripheral $\gamma\delta$ T cells may contribute greatly to the inhibition of cancer.

Pharmacological effects of *Lentinus edodes*

Lentinus edodes (Shiitake) is a common edible mushroom found in both Japan and China, and has been cultivated in Japan for around 300 years. The part of the mushroom used as food consists of the reproductive body, also referred to as the fruiting body of fungi, which produces spores for reproduction, while the vegetative body includes hyphae which produce mycelia extending into a growing area such as soil or logs.

Lentinus edodes has long been believed to have some effect against a variety of diseases and symptoms, but it is only relatively recently that any pharmacological effect has been elucidated. Various effects of extract of *Lentinus edodes* mycelium are reported, these include the inhibition of oncogenesis in the large bowel and liver and the growth of transplanted tumor cells and increased survival of animals in carcinogenesis experiments in rats and mice (N. Sugano et al., *Cancer Letter*, 27:1, 1985; Y. Suzuki et al., *Journal of the Japan Society of Coloproctology*, 43:178, 1990, etc.); mitogenic activity (T. Tabata et al., *Immunopharmacology*, 24:57, 1992; Y. Hibino et al., *Immunopharmacology*, 28:77, 1994, etc.); enhanced antibody production and inhibitory effects against immunological hepatocyte damage caused by ADCC (antibody-dependent cell-mediated cytotoxicity) (Y. Mizoguchi et al., *Journal of Hepato-Biliary-Pancreatic Study*, 15:127, 1987).

These findings have acted as a catalyst for concentrated research into the pharmacological effects of ingredients of *Lentinus edodes* in the medical and pharmaceutical field. As a result, researchers have 5 discovered that some of the constituents of *Lentinus edodes* can be used in the treatment of cancer or other diseases by restoring immune function in humans, and further that such constituents may also inhibit the onset of cancer.

In accomplishing the present invention the inventors 10 have aimed to further elucidate pharmacological effects of an extract of *Lentinus edodes* mycelium, and also to search for new applications of the extract in the form of drugs, foods, drinks, feeds, etc.

One object of the present invention is to develop and 15 provide a $\gamma\delta$ T cell activity enhancer and therefore an immunopotentiator such as an antitumor agent, therapeutic agent against bacterial infections and therapeutic agent against viral infections containing an extract of *Lentinus edodes* mycelium.

20 Another object of the present invention is to use a $\gamma\delta$ T cell activity enhancer containing an extract of *Lentinus edodes* mycelium or an immunopotentiator containing an extract of *Lentinus edodes* mycelium to treat a tumor in a subject.

25

DISCLOSURE OF THE INVENTION

As a result of intensive studies aimed at solving the above problems, the present invention has been accomplished

on the basis of the finding that an extract of *Lentinus edodes* mycelium exhibits an effect of remarkably enhancing the activity of $\gamma\delta$ T cells.

Accordingly, the present invention has been developed 5 and provided $\gamma\delta$ T cell activity enhancers and therefore immunopotentiators such as antitumor agents or therapeutic agents against bacterial or viral infections containing the extract of *Lentinus edodes* mycelium.

The present invention also provides methods developed 10 for treating tumor, bacterial infections and viral infections using the extract of *Lentinus edodes* mycelium.

$\gamma\delta$ T cell activity enhancers or immunopotentiators of the present invention may be in the form of a pharmaceutical composition containing the extract of 15 *Lentinus edodes* mycelium and optionally a pharmaceutically acceptable carrier.

$\gamma\delta$ T cell activity enhancers or immunopotentiators of the present invention may be administered in the form of injection or oral, mucosal, gastrointestinal or 20 percutaneous formulation.

$\gamma\delta$ T cell activity enhancers or immunopotentiators of the present invention may also be in the form of a food, drink or feed.

25 BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows that the proportion of $\gamma\delta$ T cells in peripheral blood increases following administration of the extract of *Lentinus edodes* mycelium of the present

invention.

FIG. 2 shows the results of flow cytometric analysis of $\gamma\delta$ T cells before and after administration of the extract of *Lentinus edodes* mycelium.

5 FIG. 3 shows that the proportion of $\alpha\beta$ T cells in peripheral blood decreases following administration of the extract of *Lentinus edodes* mycelium of the present invention.

10 THE MOST PREFERRED EMBODIMENTS OF THE INVENTION

An extract of *Lentinus edodes* mycelium used for enhancing the activity of $\gamma\delta$ T cells according to the present invention refers to an extract obtained by crushing and decomposing mycelia grown from *Lentinus edodes* cultured 15 on a solid medium, or a solid medium itself containing *Lentinus edodes* mycelia in the presence of water and an enzyme.

An extract of *Lentinus edodes* mycelium used herein is preferably obtained by, but not limited to, the following 20 process. *Lentinus edodes* spawn is inoculated on a solid medium based on bagasse (sugar cane residue) and defatted rice bran to grow mycelia, and then the solid medium containing the grown mycelia is delignified so that 30% by weight or less is able to pass through a 12-mesh sieve. To 25 this delignified solid medium are added water and one or more enzymes selected from cellulase, protease or glucosidase while maintaining said solid medium at a temperature of 30-55°C, and said solid medium is crushed

and ground in the presence of said enzyme so that at least 70% by weight of bagasse fiber is able to pass through a 12-mesh sieve. Then, the temperature is raised to 95°C to ensure inactivation of the enzyme and sterilization, and 5 the resulting suspension is filtered to give an extract of *Lentinus edodes* mycelium. The extract of *Lentinus edodes* mycelium as prepared above may be directly used in $\gamma\delta$ T cell activity enhancers of the present invention, but conveniently concentrated and freeze-dried into powder to 10 be stored and used in various forms. The freeze-dried product is a brown powder with hygroscopic characteristics and has a peculiar taste and odor.

The extract of *Lentinus edodes* mycelium was tested for its *in vivo* $\gamma\delta$ T cell activity-enhancing effect by the 15 method described in the examples below, and it will be seen from these examples that it has a remarkable *in vivo* $\gamma\delta$ T cell activity-enhancing effect.

$\gamma\delta$ T cell activity enhancers of the present invention are effective for treating and/or preventing tumor induced 20 by tumor cells to which $\gamma\delta$ T cells are cytotoxic. $\gamma\delta$ T cell activity enhancers of the present invention are characterized in that they enhance the activity of $\gamma\delta$ T cells and thereby lead to the destruction of tumor cells under the action of the activated $\gamma\delta$ T cells, rather than 25 having a direct action on specific tumor cells.

Consequently, tumor cells to be treated with $\gamma\delta$ T cell activity enhancers of the present invention may be not only malignant tumor cells but also benign tumor cells, and are

not limited to specific tumor cells. In addition, $\gamma\delta$ T cell activity enhancers containing the extract of *Lentinus edodes* mycelium and optionally a pharmaceutically acceptable carrier can be used in the form of both 5 therapeutic and prophylactic compositions.

$\gamma\delta$ T cell activity enhancers of the present invention can also be used as therapeutic and/or prophylactic compositions for bacterial or viral infections. $\gamma\delta$ T cell activity enhancers of the present invention are intended to 10 enhance the activity of $\gamma\delta$ T cells and thereby to remove infecting bacteria or viruses from the patient rather than to directly act on specific bacteria or viruses. Bacterial or viral diseases that can be treated with $\gamma\delta$ T cell activity enhancers of the present invention include such 15 diseases as , but are not limited to, those caused by *Mycobacterium* spp. *Listeria monocytogenes*, hepatitis viruses (types A, B and C), human immunodeficiency virus, vaccinia virus and the like.

$\gamma\delta$ T cell activity enhancers of the present invention 20 in the form of a therapeutic and/or prophylactic composition are administered most preferably via the oral route, but may also be administered via intravenous, intraperitoneal, subcutaneous, intramuscular, nasal, percutaneous or other route. Dosage forms suitable for 25 oral administration include, but are not limited to, tablets, capsules, powders, granules, solutions, syrups, etc. Dosage forms suitable for nasal or percutaneous administration include, but not limited to, cataplasms,

patches, etc.

Pharmaceutically acceptable carriers include, but are not limited to, suitable excipients, binders, disintegrators, lubricants, flavoring agents, colorants, 5 solubilizers, suspending agents, coating agents or the like known in the art.

Pharmaceutically acceptable carriers that can be optionally mixed in $\gamma\delta$ T cell activity-enhancing formulations of the present invention include, but are not 10 limited to excipients such as lactose, dextrose, starch, crystalline cellulose; binders such as starch, gelatin, methyl cellulose, polyvinylpyrrolidone; disintegrators such as starch, calcium carboxymethylcellulose, carboxymethyl starch; lubricants such as talc, stearates; coating agents 15 such as sucrose, talc, gelatin; as well as various brighteners, flavoring agents, colorants, corrigents, solubilizers, stabilizers, suspending agents, absorbefacients or the like known in the art depending on the purpose. For use as injections, various diluents 20 commonly used in this field of art such as water or ethyl alcohol can be used.

The dose of $\gamma\delta$ T cell activity enhancers of the present invention is determined by physicians taking into account the age, weight and condition of the subject, the 25 route of administration and other factors. The dose is not strictly limited because the extract of *Lentinus edodes* mycelium contained in $\gamma\delta$ T cell activity enhancers of the present invention is highly safe, and has been

traditionally ingested as a component of food. For example, the extract of *Lentinus edodes* mycelium is normally administered preferably at a dose of 100 mg - 10000 mg several times (about 2-3 times) daily (a total of 200 mg - 5 30000 mg daily), more preferably 500 mg - 5000 mg three times daily (a total of 1500 mg - 15000 mg daily), most preferably 1000 mg - 1500 mg three times daily (a total of 3000 mg - 4500 mg daily). It may be administered in combination with other antitumor agents.

10 $\gamma\delta$ T cell activity enhancers of the present invention can be provided in a dosage form also suitable for adoptive immunotherapy for treating tumor. Adoptive immunotherapy refers to a type of anti-tumor therapy intended to kill tumor cells by transferring into a subject sensitized cells, 15 normally lymphocytes. In the case of the present invention, $\gamma\delta$ T cells are initially isolated from peripheral blood of the subject, and the isolated $\gamma\delta$ T cells are activated in vitro by a $\gamma\delta$ T cell activity enhancer of the present invention, and then the activated $\gamma\delta$ T cells are returned 20 into the subject. As a result, tumor cells in the subject can be destroyed by the action of the activated $\gamma\delta$ T cells.

25 $\gamma\delta$ T cell activity enhancers of the present invention may be the extract of *Lentinus edodes* mycelium itself or pharmaceutical or veterinary compositions comprising a $\gamma\delta$ T cell activity enhancer containing the extract of *Lentinus edodes* mycelium and a pharmaceutically acceptable carrier.

30 $\gamma\delta$ T cell activity enhancers of the present invention can also be provided in the form of a food. Preferred

forms of food include powders, granules, pastes, jellies, etc. Granules desirably are supplemented with sugars such as lactose to add a sweet taste. $\gamma\delta$ T cell activity enhancers of the present invention can also be provided in 5 the form of a drink. These foods or drinks may be supplemented with vitamins, minerals such as calcium, alcohols, deodorants such as polyphenols in addition to the extract of *Lentinus edodes* mycelium. These foods or drinks include the categories of specific health foods, medical 10 foods or the like.

$\gamma\delta$ T cell activity enhancers of the present invention can also be provided in the form of a feed or feed additive. $\gamma\delta$ T cell activity enhancers of the present invention can be used as a feed or feed additive for domestic animals to 15 treat and/or prevent tumor occurring in domestic animals or to treat and/or prevent bacterial or viral infections in domestic animals. As a result, the amount of therapeutic agents such as antibiotics currently used can be reduced, thereby reducing farming costs. Another advantage is that 20 the period during which shipment of animals is suspended due to the administration of antibiotics can be shortened.

In vivo $\gamma\delta$ T cell activity-enhancing effect was tested in human subjects as follows. Human subjects initially received 3.6 g of the extract of *Lentinus edodes* mycelium 25 bulk powder daily for 7 days (a total of 25.2 g). Then, the proportion of $\gamma\delta$ T cells in peripheral blood after administration of the extract of *Lentinus edodes* mycelium was determined by flow cytometry as compared with the

proportion before administration.

The following examples, which further illustrate the present invention, should not be taken as limiting the the scope of the invention thereto. Various changes and 5 modifications can be made by those skilled in the art and such changes and modifications are also included in the scope of the present invention.

EXAMPLES

10 Example 1: Preparation of an extract of *Lentinus edodes* mycelium

A solid medium consisting of 90 parts by weight of bagasse and 10 parts by weight of rice bran was soaked with an appropriate amount of pure water, and then inoculated 15 with *Lentinus edodes* spawn and allowed to stand in an incubator at controlled temperature and humidity to grow mycelia. After mycelia spread over the solid medium, the bagasse base was delignified so that 24% by weight or less may pass through a 12-mesh sieve. To 1.0 kg of this 20 delignified medium were added 3.5 L of pure water and 2.0 g of purified cellulase while maintaining the solid medium at 40°C to prepare a medium-containing mixture.

Then, the medium-containing mixture was circulated by a variable speed gear pump, during which the solid medium 25 was crushed and ground under the gears for about 200 minutes so that about 80% by weight of bagasse fiber may pass through a 12-mesh sieve. The medium-containing mixture was crushed and ground while the temperature of

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said mixture was gradually increased. Then, the medium-containing mixture was further heated to 90°C and allowed to stand for 30 minutes. This heating to 90°C ensured inactivation of the enzyme and sterilization. The 5 resulting medium-containing mixture was filtered through a 60-mesh filter cloth to give an extract of *Lentinus edodes* mycelium solution, which was concentrated and then freeze-dried into an extract of *Lentinus edodes* mycelium bulk powder.

10 The extract of *Lentinus edodes* mycelium as prepared above contained 25.3% (w/w) carbohydrates determined by the phenol-sulfuric acid method, 19.7% (w/w) proteins determined by the Lowry method and 2.6% (w/w) polyphenols determined by the Folin-Denis method using gallic acid as 15 standard. The extract of *Lentinus edodes* mycelium further contains 8% crude fat, 22% crude ash and about 20% soluble nitrogen-free materials other than carbohydrates.

The extract of *Lentinus edodes* mycelium had a sugar composition (%) as follows: Xyl 15.2, Ara 8.2, Man 8.4, Gul 20 39.4, Gal 5.4, GlcN 12.0, GluUA 11.3.

Example 2: In vivo $\gamma\delta$ T cell activity-enhancing test of the extract of *Lentinus edodes* mycelium

Three human subjects (subjects A-C) received orally 25 3.6 g/day of the extract of *Lentinus edodes* mycelium bulk powder daily for 7 days (a total of 25.2 g). After the period of administration of the extract of *Lentinus edodes* mycelium, peripheral blood was collected from the human

subjects. The proportion of $\gamma\delta$ T cells in peripheral blood collected after administration was determined by flow cytometry as compared with the proportion in peripheral blood collected before administration. The results are
5 shown in Figs. 1 and 2.

In all of the three subjects, the proportion of $\gamma\delta$ T cells in peripheral blood increased by an average of 40% or more after administration of the extract of *Lentinus edodes* mycelium as compared with before administration.

10

Table 1: Increase of the proportion of $\gamma\delta$ T cells in peripheral blood after administration

| | Subject A | Subject B | Subject C | Mean \pm SEM |
|----------|-----------|-----------|-----------|---------------------|
| Increase | 124.39% | 146.15% | 150.00% | 140.18% \pm 7.97% |

15

Peripheral blood collected from the same subject before and after administration was tested for other markers than $\gamma\delta$ T cells. As a result, the extract of *Lentinus edodes* mycelium showed no proliferative activity
20 on $\alpha\beta$ T cells and on average the proportion of $\alpha\beta$ T cells decreased (Fig. 3).

INDUSTRIAL APPLICABILITY

$\gamma\delta$ T cell activity enhancers containing the extract of
25 *Lentinus edodes* mycelium of the present invention were found to actually activate $\gamma\delta$ T cells. Thus, $\gamma\delta$ T cell activity enhancers of the present invention can be used for preventing or treating tumor, bacterial infections and

viral infections because they have the effect of protecting living bodies against tumor, bacterial infections and viral infections by inducing cytotoxic activity against tumor cells, antibacterial activity and antiviral activity of $\gamma\delta$ T cells. Moreover, $\gamma\delta$ T cell activity enhancers of the present invention are suitable for wide industrial application because they can be used safely without side effects.

They can also be used in domestic animals with bacterial and/or viral infections to reduce the amount of therapeutic agents currently used such as antibiotics, thereby reducing costs for raising. They also have the advantage that the period during which shipment is suspended can be shortened because antibiotics are not used.

CLAIMS

1. A $\gamma\delta$ T cell activity enhancer containing an extract of *Lentinus edodes* mycelium.
2. A pharmaceutical or veterinary $\gamma\delta$ T cell activity enhancer comprising an extract of *Lentinus edodes* mycelium and a pharmaceutically acceptable carrier.
3. The $\gamma\delta$ T cell activity enhancer of claim 1 or 2 for oral administration.
4. The $\gamma\delta$ T cell activity enhancer of claim 1 in the form of a food.
5. The $\gamma\delta$ T cell activity enhancer of claim 1 in the form of a drink.
6. The $\gamma\delta$ T cell activity enhancer of claim 1 in the form of a feed.
7. The $\gamma\delta$ T cell activity enhancer of claim 1 or 2 for injection or percutaneous absorption.
8. The $\gamma\delta$ T cell activity enhancer of claim 1 for *in vitro* activating $\gamma\delta$ T cells collected from a subject.
9. The $\gamma\delta$ T cell activity enhancer of any one of claims 1 to 8 used for treating tumor.
10. The $\gamma\delta$ T cell activity enhancer of any one of claims 1 to 8 used for preventing or treating a bacterial infection or a viral infection.
11. A method for treating a tumor of a subject by administering *in vivo* the $\gamma\delta$ T cell activity enhancer of claim 9.
12. A method for preventing or treating a bacterial infection or a viral infection in a subject by

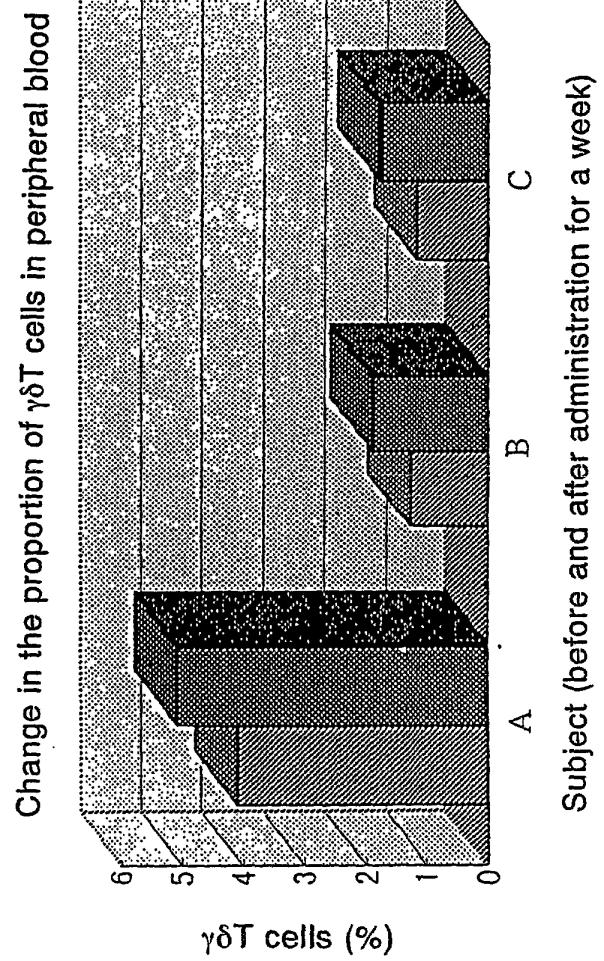
administering in vivo the $\gamma\delta$ T cell activity enhancer of claim 10.

13. A use of an extract of *Lentinus edodes* mycelium for the preparation of a $\gamma\delta$ T cell activity enhancer.

ABSTRACT

The present invention develops and provides $\gamma\delta$ T cell activity enhancers and therefore immunopotentiators containing an extract of *Lentinus edodes* mycelium for use 5 in the treatment of tumor or the treatment and/or prevention of bacterial infections or viral infections by taking advantage of the effect that the extract of *Lentinus edodes* mycelium has in remarkably enhancing the activity of $\gamma\delta$ T cells.

FIG. 1



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FIG. 2

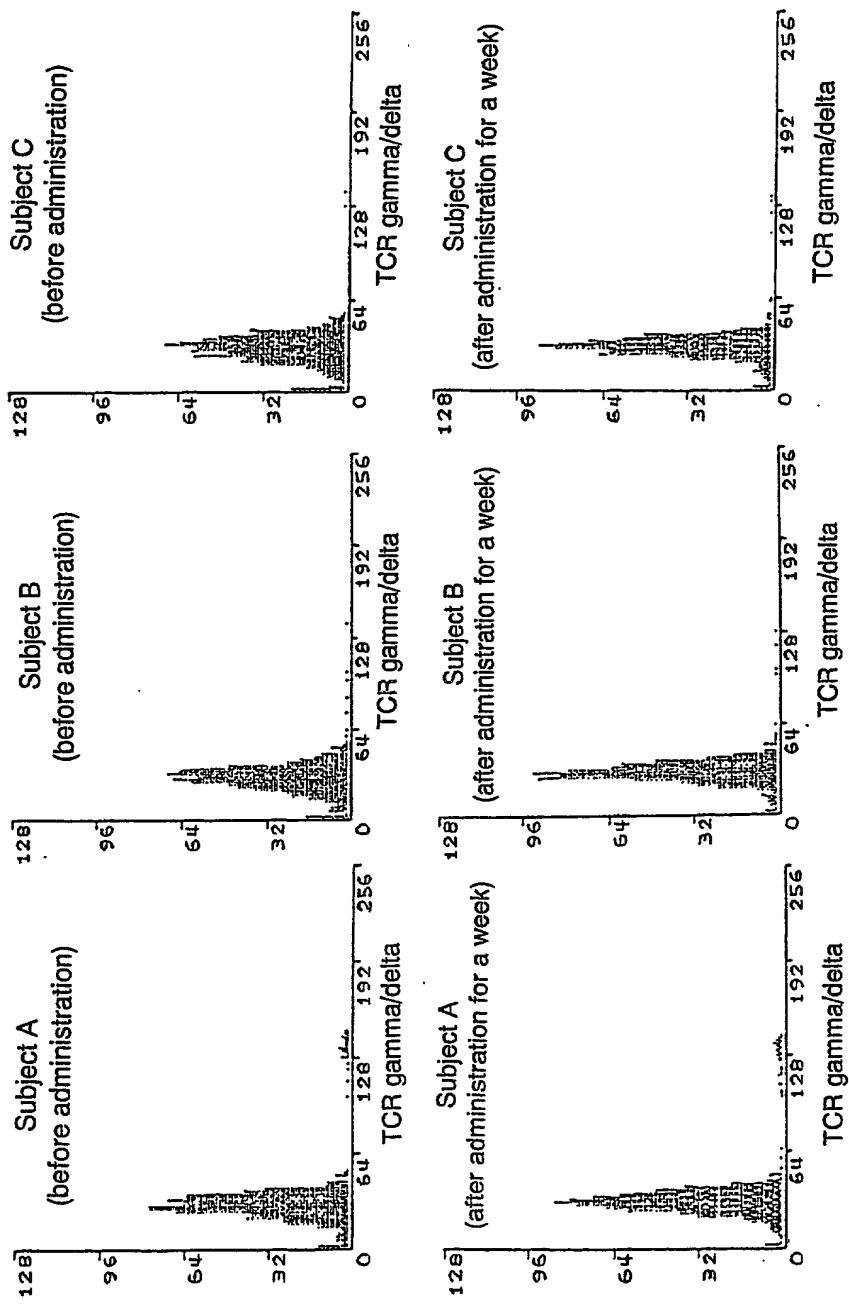
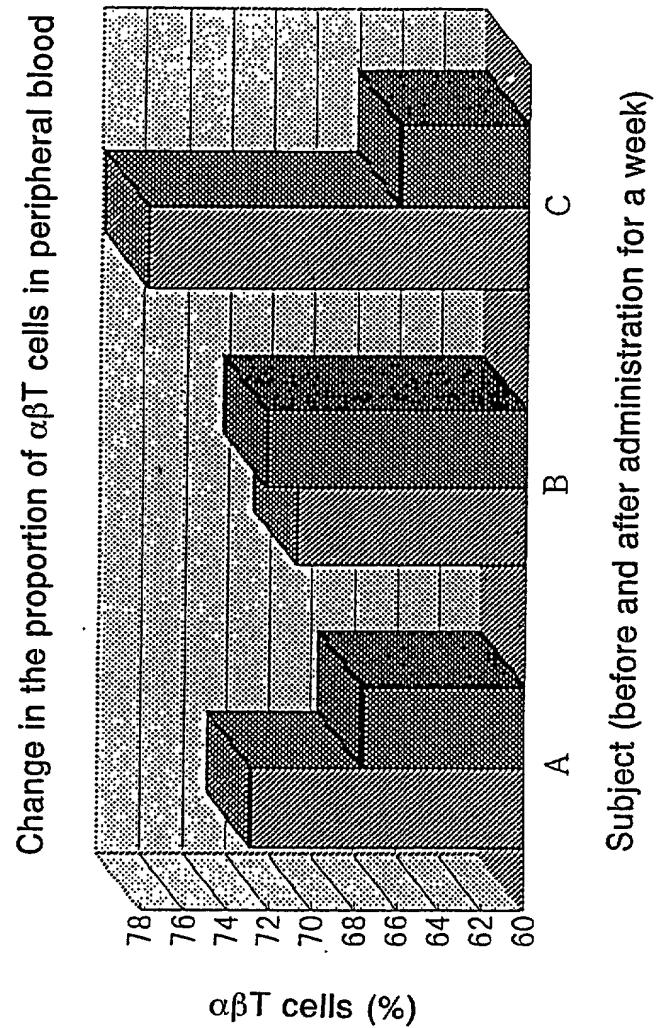


FIG. 3



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As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

$\gamma\delta$ T CELL IMMUNOACTIVITY ENHANCERS CONTAINING EXTRACT OF
LENTINUS EDODES MYCELIUM

Fill in Appropriate
Information -

For Use Without
Specification
Attached:



the specification of which is attached hereto. If not attached hereto,

the specification was filed on _____ as
United States Application Number _____; and /or

the specification was filed on November 26, 1999 as PCT
International Application Number PCT/JP99/06617; and was
amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

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Prior Foreign Application(s)

| Priority (Number) | Claimed Country | Priority (Month/Day/Year Filed) | Priority Claimed Yes | Priority Claimed No |
|----------------------|--------------------|------------------------------------|-------------------------------------|---------------------------|
| 337822/1998 | Japan | 11/27/98 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| (Number) | (Country) | (Month/Day/Year Filed) | <input type="checkbox"/> | <input type="checkbox"/> |
| (Number) | (Country) | (Month/Day/Year Filed) | <input type="checkbox"/> | <input type="checkbox"/> |
| (Number) | (Country) | (Month/Day/Year Filed) | <input type="checkbox"/> | <input type="checkbox"/> |
| (Number) | (Country) | (Month/Day/Year Filed) | <input type="checkbox"/> | <input type="checkbox"/> |
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| Country | Application No. | Date of Filing (Month/Day/Year) |
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| (Application Number) | (Filing Date) | (Status - patented, pending, abandoned) |
|----------------------|---------------|---|
| | | |
| | | |

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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Full Name of Third Inventor, if any:

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Full Name of Fourth Inventor, if any

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